UNIVERSITY of WASHINGTON

APPROVAL OF NEW PROTOCOL SUBMISSION

September 1, 2020

Dear Dr. Hampe,

This email serves as written notice of animal use approval by the Institutional Animal Care and Use Committee (IACUC).

*To help us better serve you, please take this **3 question survey** about your experience with the review process.*

Type of Review:	Designated Member Review
Short Title of Protocol:	4189-04: B cell tracking
Investigator:	Christiane S. Hampe
HoverBoard ID:	PROTO201900104

Please note the approval and expiration date listed. All animal use protocols must be renewed annually from the date of IACUC approval, independent of project or funding dates. Please refer to the assigned protocol number for all animal orders and future correspondence with the IACUC.

Protocol Approval Dates: 9/1/2020 to 8/31/2023

Next Triennial Expiration Date: 8/31/2023

If you have any questions, contact OAWRSS at oawrss@uw.edu.

Sincerely,

Office of Animal Welfare

DO NOT USE FOR GRANT/CONTRACT VERIFICATION

OFFICE OF ANIMAL WELFARE

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Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

From: J.Preston Van Hooser <jpvh@uw.edu>
Sent: Friday, November 6, 2020 11:58 AM
To: Bob Ennes
Subject: Fw: 4189-04: B cell tracking [RE 11/5/2020 PUBLIC RECORDS REQUEST - RISE FOR ANIMALS' EMAIL 2 OF 6

Forwarding Email 2 of 6 re UW IACUC# 4189-04 (PI: Christiane Hampe)

From: J.Preston Van Hooser
Sent: Monday, August 17, 2020 7:45 PM
To: Christiane S. Hampe <champe@uw.edu>
Subject: Re: 4189-04: B cell tracking

Thanks Chris. Also, I updated the Funding Page in HoverBoard an Exp 001 Q5 and Q10a in an attempt to be helpful per earlier vet preview comments. I'm short I removed Exp 002 and incorporated it into Exp 001. Please take a look to make sure I didn't good anything up and change as/if necessary and then submit it back. We should be getting close to sending this off to the committee for final review and approval.

Best,
Preston

Sent from my iPhone

> On Aug 17, 2020, at 5:51 PM, Christiane S. Hampe <champe@uw.edu> wrote:
>
> Hi Preston,
>
> I attached the Immusoft proposal. The internal eGC1 number is A161417.
>
> Thanks for taking care of this!!
>
> Chris
>
> > On Aug 17, 2020, at 5:36 PM, J.Preston Van Hooser <jpvh@uw.edu> wrote:
> >
> > Hi Chris,
> >
> > Can you email me a copy of the Immusoft grant and internal eGC1 number that you created for this (per your recent response to Reviewer Notes in HoverBoard for your new protocol noted above). I'm not finding anything in SAGE or SPAERC.
> >
> > Thank you,
> > Preston
> >
> > J. Preston Van Hooser
> > Review Scientist & Compliance Manager

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Chair, Dare 2 Care (D2C) Compassion in Science Committee
Office of Animal Welfare

Health Sciences Building Box 357160
1705 NE Pacific Street / Seattle, WA 98195-7160
206.616.8417 / mobile 206.940.3176 / fax 206.616.1297
jpvh@uw.edu / oaw.washington.edu

<image001.gif>

<image004.png>

Dare 2 Care... | explore UW's Compassion Fatigue Program

<osp.docx>

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From: J.Preston Van Hooser <jpvh@uw.edu>
Sent: Friday, November 6, 2020 12:01 PM
To: Bob Ennes
Subject: Fw: animal protocol OSP [RE 11/5/2020 PUBLIC RECORDS REQUEST - RISE FOR ANIMALS] EMAIL 5 OF 6

Forwarding Email 5 of 6 re UW IACUC# 4189-04 (PI: Christiane Hampe)

From: J.Preston Van Hooser
Sent: Monday, August 31, 2020 12:03 PM
To: Chris Hampe <chris.hampe@immusoft.com>
Subject: RE: animal protocol OSP

Hi Chris,

Thanks for your email. For clarification, per our discussion, you don't' need an eGC1 for the funding source. The animal use protocol that we prepared is under committee review. The protocol doesn't go to OSP, but rather the IACUC only. I hope this helps clarify the funding question that was raised.

Best,
Preston

J. Preston Van Hooser
Review Scientist & Compliance Manager
Chair, Dare 2 Care (D2C) Compassion in Science Committee
Office of Animal Welfare

Health Sciences Building Box 357160
1705 NE Pacific Street / Seattle, WA 98195-7160
206.616.8417 / mobile 206.940.3176 / fax 206.616.1297
jpvh@uw.edu / oaw.washington.edu

Dare 2 Care… | explore UW's Compassion Fatigue Program

-----Original Message-----
From: Chris Hampe <chris.hampe@immusoft.com>
Sent: Friday, August 28, 2020 4:10 PM
To: J.Preston Van Hooser <jpvh@uw.edu>
Subject: animal protocol OSP

Hi Preston,

this is a follow-up email regarding the animal protocol. You had mentioned earlier this week that it may not be necessary for me to submit the protocol through OSP. Do you have more information regarding this? And what else needs to be done on the IACUC protocol?

Many thanks and have a great weekend!

Chris

Chris Hampe, PhD
Director of Research

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Immusoft Corporation
454 N 34th St.
Seattle WA 98103
chris.hampe@immusoft.com
Cell phone: +1-206-554-9181

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Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

From: J.Preston Van Hooser <jpvh@uw.edu>
Sent: Friday, November 6, 2020 12:01 PM
To: Bob Ennes
Subject: Fw: Immusoft questions [RE 11/5/2020 PUBLIC RECORDS REQUEST - RISE FOR ANIMALS] EMAIL 4 OF 6
Attachments: image004.gif; image005.png; image001.jpg

Forwarding Email 4 of 6 re UW IACUC# 4189-04 (PI: Christiane Hampe)

From: J.Preston Van Hooser
Sent: Tuesday, August 25, 2020 7:17 AM
To: Emily W. Clark <ewilkins@uw.edu>
Cc: Patrick Cory <pcory@uw.edu>
Subject: Re: Immusoft questions

Hi Patrick and Emily,

Yes, I am aware in that when I asked her to complete the Funding Page for her new protocol, she eventually listed Immusoft as the sponsor of which I then had to ask for a copy of the grant and associated eGC1.

I will follow-up with OSP and Chris to figure out what is going on. This one has been very confusing.

Patrick - I'll circle back as I am able.

Best,
Preston

Sent from my iPhone

> On Aug 24, 2020, at 9:20 PM, Emily W. Clark <ewilkins@uw.edu> wrote:
>
> Hi Patrick,
>
> Thanks for your message. I'm not sure I completely follow what's happening, but I do know of a few other PIs that seem to have a similar arrangement (animal work done at UW with outside funds that do not generate an eGC1 – the M3 Biotechnology group, for example). I know Dr. Hampe has a new IACUC protocol that is currently under review. I'm cc'ing Preston Van Hooser, who is Dr. Hampe's OAW Scientific Liaison. Preston, do you have any insight into this new project?
>
> Thanks again,
> Emily
>
> **Emily W. Clark, PhD**
> Review Scientist
> Office of Animal Welfare Research Support Services
>
> Health Sciences Building Box 357160

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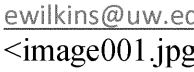
1705 NE Pacific Street Seattle, WA 98195-7160
206.685.7475 fax 206.616.1297
ewilkins@uw.edu / oaw.washington.edu


From: Patrick Cory <pcory@uw.edu>
Sent: Monday, August 24, 2020 5:31 PM
To: Emily W. Clark <ewilkins@uw.edu>
Subject: FW: Immusoft questions

Hi Emily,

Dr. Hampe (an affiliate in Department of Medicine) is seeking UW IACUC approval for a project for which he attached a statement of work and budget. I was under the assumption that IACUC approval requires an eGC1. Would there be IACUC approval outside of an eGC1, for external fee-for-service type work?

Thanks,
Patrick

Patrick Cory, CRA
Administrator
Department of Comparative Medicine
HSB I-422, Box 357340
(206) 685-3255 / washington.edu


From: Lisa Spencer <spence57@uw.edu>
Sent: Monday, August 24, 2020 11:14 AM
To: Patrick Cory <pcory@uw.edu>
Subject: Immusoft questions

Hi Pat,

Can you give me any background/guidance regarding the Immusoft project? It almost sounds like Chris Hampe isn't going to make an award to UW – it seems like he plans to pay for work that will be done at UW. Kind of like a fee for service. What's your understanding?

Is that something Comp Med does for Affiliates?

Thanks,

Lisa

Lisa Spencer
Budget/Fiscal Analyst Lead
Administrative Business Center (ABC)

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Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

spence57@uw.edu
Direct line: 206.685.8747
ABC general line: 206.685.2508

<image005.png>

From: Chris Hampe <chris.hampe@immusoft.com>
Sent: Monday, August 24, 2020 11:01 AM
To: Lisa Spencer <spence57@uw.edu>
Subject: Re: New application

Hi Lisa,

First: 8/29 is not a hard deadline.

Here are my responses to the other questions:

1) I am employed by Immusoft and Immusoft will pay for the project, so that there is no award document associated with the project.
2) The work will be carried out by Comparative Medicine. Robert Hunter and Charlie Hsu are my contacts at the department.
3) the money for the project has been raised by Immusoft and is not provided by another entity.
4) The work will be carried out at the animal facilities of the UW.
5) No human subjects are involved.
6) the project requires use of animals.
7) all this is part to get the project approved by IACUC, and I need no further documents.
8) I attached the SOW, budget, and budget justification.

Many thanks,
Chris

Chris Hampe, PhD
Director of Research
Immusoft Corporation
454 N 34th St.
Seattle WA 98103
chris.hampe@immusoft.com
Cell phone: +1-206-554-9181

> On Aug 24, 2020, at 9:57 AM, Lisa Spencer <spence57@uw.edu> wrote:
>
> Hi Chris,
>
> I have several questions:
>
> 1. Is there already an award document?
> 2. Who will the UW PI be?
> 3. Has this funding been awarded to Immusoft by another entity (i.e., NIH, NSF, DoD, etc.)?
> 4. Where will the work be done?

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5. Will there be any Human Subjects?
6. Will there be any Animal Subjects?
7. Do you need any other documents besides the ones we'll need (listed below)
8. We have an internal 3 day deadline to route this through OSP – is 8/29/20 a hard deadline or do we have a little wiggle room?

We'll need the following to complete the award document:

1. A budget spreadsheet
2. A budget Justification
3. Scope of Work

Thanks!

Lisa

Lisa Spencer
Budget/Fiscal Analyst Lead
Administrative Business Center (ABC)
spence57@uw.edu
Direct line: 206.685.8747
ABC general line: 206.685.2508

<image001.png>

From: Chris Hampe <chris.hampe@immusoft.com>
Sent: Monday, August 24, 2020 9:00 AM
To: Lisa Spencer <spence57@uw.edu>
Subject: Re: New application

Hi Lisa,

Thanks, I assigned you access to read and write.
Please let me know if you have questions.

Chris

Chris Hampe, PhD
Director of Research
Immusoft Corporation
454 N 34th St.
Seattle WA 98103
chris.hampe@immusoft.com
Cell phone: +1-206-554-9181

On Aug 24, 2020, at 7:52 AM, Lisa Spencer <spence57@uw.edu> wrote:

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Hi Chris,

If you can give me access to your eGC1 I'll be happy to review it for you.

Thanks,

Lisa

Lisa Spencer
Budget/Fiscal Analyst Lead
Administrative Business Center (ABC)
spence57@uw.edu
Direct line: 206.685.8747
ABC general line: 206.685.2508

<image001.png>

From: Chris Hampe <chris.hampe@immusoft.com>
Sent: Friday, August 21, 2020 10:57 AM
To: Lisa Spencer <spence57@uw.edu>
Subject: New application

Hello Lisa,

I was referred to you by Patrick Cory at Comparative Medicine.

I am an affiliated Research Associate Professor at the Department of Medicine at the UW. I am working at a biotech company in Seattle (Immusoft) and we plan to carry out an animal project with the Department of Comparative Medicine at the UW. The research is going to be funded through Immusoft.

I am in the process of completing the IACUC application for the project and apparently I need to set up the award through OSP. I have generated (to the best of my abilities) an internal eGC1 (A161417) and attached a copy of the proposal to this email. I am not sure how detailed the proposal has to be, and whether you need biosketches, budget and budget justification.

Robert Hunter at Comparative Medicine has already signed the SFI.

I am not clear about the steps involved and hope that you can guide me through this.

Please contact me at my email (chris.hampe@immusoft.com), or by phone: 206-554-9181.

Many thanks,

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Chris

Chris Hampe, PhD
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454 N 34th St.
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UW Medicine

UW SCHOOL OF MEDICINE

ADMINISTRATIVE BUSINESS CENTER

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UNIVERSITY of WASHINGTON

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From: J.Preston Van Hooser <jpvh@uw.edu>
Sent: Friday, November 6, 2020 12:02 PM
To: Bob Ennes
Subject: Fw: Immusoft questions [RE 11/5/2020 PUBLIC RECORDS REQUEST - RISE FOR ANIMALS] EMAIL 6 OF 6

Forwarding Email 6 of 6 re UW IACUC# 4189-04 (PI: Christiane Hampe)

From: J.Preston Van Hooser
Sent: Monday, August 31, 2020 12:08 PM
To: Patrick Cory <pcory@uw.edu>
Subject: RE: Immusoft questions

Hi Patrick,

After further discussion with Dr. Hampe, and DCM IVS (Bob Hunter), she will not need an eGC1 number as no funds will be routing through OSP. DCM will bill Immusoft as they would following current practices for fee for service.

If you have any questions, please feel free to give me a call. This one was more confusing than it needed to be but after much back-and-forth with Chris, I think I finally understand what she is doing.

Best,
Preston

J. Preston Van Hooser
Review Scientist & Compliance Manager
Chair, Dare 2 Care (D2C) Compassion in Science Committee
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Direct line: 206.685.8747
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<image005.png>

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Thanks!

Lisa

Lisa Spencer
Budget/Fiscal Analyst Lead

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spence57@uw.edu
Direct line: 206.685.8747
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To: Lisa Spencer <spence57@uw.edu>
Subject: Re: New application

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Chris

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Subject: New application

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Many thanks,
Chris

Chris Hampe, PhD
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From: J.Preston Van Hooser <jpvh@uw.edu>
Sent: Friday, November 6, 2020 11:59 AM
To: Bob Ennes
Subject: Fw: new award [RE 11/5/2020 PUBLIC RECORDS REQUEST - RISE FOR ANIMALS] EMAIL 3 OF 6

Forwarding Email 3 of 6 re UW IACUC# 4189-04 (PI: Christiane Hampe)

From: J.Preston Van Hooser <jpvh@uw.edu>
Sent: Thursday, August 20, 2020 9:32 AM
To: Office Of Sponsored Programs <osp@uw.edu>; Selesteen Jimenez <sjimenez@uw.edu>
Cc: Chris Hampe <chris.hampe@immusoft.com>; Ariadna A. Santander <filimus@uw.edu>; Tim Mhyre <tmhyre@uw.edu>
Subject: Re: new award

Thanks everyone.

Preston

J. Preston Van Hooser
Review Scientist & Compliance Manager

1705 N.E. Pacific Street, HSB Rm T-252 | Seattle, WA 98195
206-616-8417 (office) | 206-616-5664 (fax) | 206-940-3176 (mobile)

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From: Office Of Sponsored Programs <osp@uw.edu>
Sent: Thursday, August 20, 2020 9:29 AM
To: Selesteen Jimenez <sjimenez@uw.edu>
Cc: Chris Hampe <chris.hampe@immusoft.com>; J.Preston Van Hooser <jpvh@uw.edu>; Ariadna A. Santander <filimus@uw.edu>; Tim Mhyre <tmhyre@uw.edu>
Subject: RE: new award

Good Morning Selesteen,

I have forwarded these emails onto Donna Parks for assistance.

Best Regards,

Dianna Lienard
Program Coordinator/Central Operations
Office of Sponsored Programs
University of Washington
4333 Brooklyn Ave NE
Box 359472, Seattle, WA 98195-9472

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Direct: 206-543-4043
Email: osp@uw.edu
Office Hrs: M-F 8:00 AM – 5:00 PM

From: Selesteen Jimenez <sjimenez@uw.edu>
Sent: Thursday, August 20, 2020 9:18 AM
To: Office Of Sponsored Programs <osp@uw.edu>
Cc: Chris Hampe <chris.hampe@immusoft.com>; J.Preston Van Hooser <jpvh@uw.edu>; Ariadna A. Santander <filimus@uw.edu>; Tim Mhyre <tmhyre@uw.edu>
Subject: FW: new award

Hello OSP,

I noticed that the below email that is addressed to OSP was sent to our office twice this week by error. Again, I'm forwarding the below email to your office, please confer with the recipient (Chris Hampe)once you have received it. Thanks

Stay Safe,
Selesteen

Selesteen Jimenez
IACUC Program Coordinator
Box 357160
(206) 616-7486
sjimenez@uw.edu
http://depts.washington.edu/oawhome/

NOTE: Pre-review of all protocol submissions is now *required*. More information at https://uwnetid.sharepoint.com/sites/OAWRSS/OAWRSSWebsite

Into the Future … Explore UW's eIACUC Solution

From: Office of Animal Welfare <oawrss@uw.edu>
Sent: Tuesday, August 18, 2020 10:58 AM
To: Selesteen Jimenez <sjimenez@uw.edu>
Subject: FW: new award

From: Chris Hampe <chris.hampe@immusoft.com>
Sent: Monday, August 17, 2020 5:12 PM
To: Office of Animal Welfare <oawrss@uw.edu>
Subject: new award

Good morning OPS,

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Hi, I am an affiliated Research Associate Professor at the Department of Medicine at the UW. I am working at a biotech company in Seattle (Immusoft) and we plan to carry out an animal project with the Department of Comparative Medicine at the UW. The research is going to be funded through Immusoft.

I am in the process of completing the IACUC application for the project and Preston alerted me to the fact that I need to set up the award through OSP. I have generated (to the best of my abilities) an internal eGC1 (A161417) and attached a copy of the proposal to this email. I am not sure how detailed the proposal has to be, and whether you need biosketches, budget and budget justification.
I am not clear about the steps involved and hope that you can guide me through this.

Please contact me at my email (chris.hampe@immusoft.com), or by phone: 206-554-9181.

Many thanks,
Chris

Chris Hampe, PhD
Director of Research
Immusoft Corporation
454 N 34th St.
Seattle WA 98103
chris.hampe@immusoft.com
Cell phone: +1-206-554-9181

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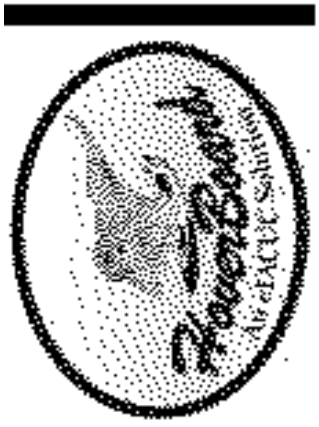

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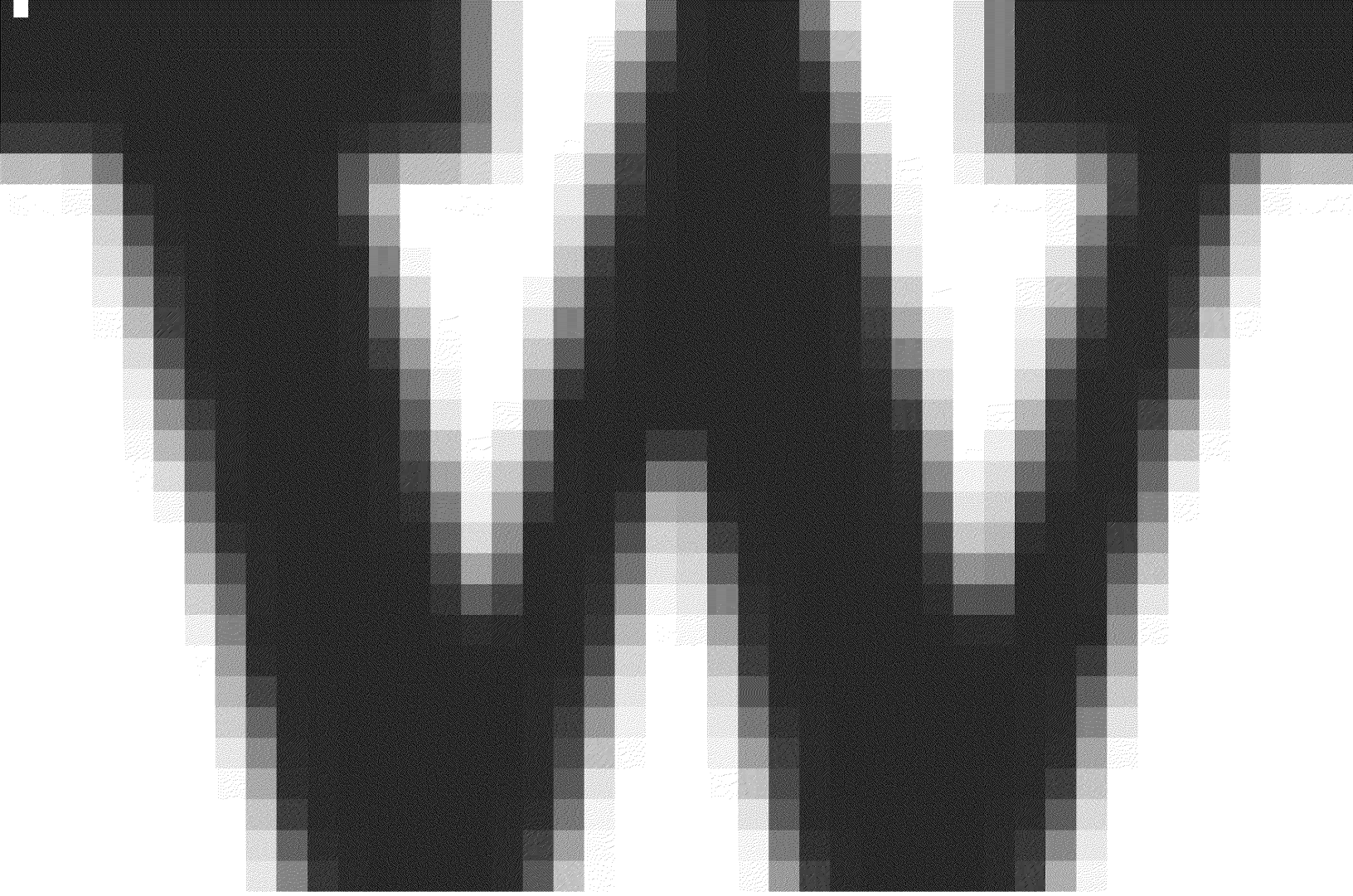

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Activity	Author	Activity Date
Major Version Incremented	Van Hooser, J.Preston	11/25/2020 11:16 AM
Amendment AMEND202001173 closed (Approved)	Van Hooser, J.Preston	11/25/2020 11:16 AM

Amendment Approved: AMEND202001173

Opened Amendment	Hampe, Christiane S.	10/21/2020 10:16 AM

Amendment: AMEND202001173

Letter Sent	Van Hooser, J.Preston	9/1/2020 12:51 PM

Correspondence_for_PROTO201900104.doc

Letter Prepared	Van Hooser, J.Preston	9/1/2020 12:50 PM

Correspondence_for_PROTO201900104.doc

Approval Period Edited	Van Hooser, J.Preston	9/1/2020 12:48 PM
Designated Member Review Submitted	Libby, Steve	9/1/2020 9:17 AM
Designated Reviewers Assigned	Huang, Stephanie W	8/26/2020 10:52 AM
Assigned to Designated Review	Huang, Stephanie W	8/26/2020 10:51 AM
Agenda Item Removed	Huang, Stephanie W	8/26/2020 10:50 AM
Assigned Portfolio ID	Huang, Stephanie W	8/26/2020 9:55 AM
Ancillary Review Submitted	Cashman, Judy L	8/24/2020 9:39 AM
Ancillary Reviews Managed	Cashman, Judy L	8/24/2020 9:38 AM
Tags Managed	Cashman, Judy L	8/24/2020 9:38 AM
OHRs attached	Cashman, Judy L	8/24/2020 9:38 AM
Private Comment Added	Libby, Steve	8/19/2020 1:14 PM

no additional questions

Meeting Assigned	Van Hooser, J.Preston	8/19/2020 9:39 AM
Pre-Review Submitted	Van Hooser, J.Preston	8/19/2020 9:39 AM
Vet Consult Submitted	Stocking, Kim	8/19/2020 7:17 AM

Do you accept the submission? yes

Vet Consult Sent	Van Hooser, J.Preston	8/18/2020 12:53 PM

Hi Kim,
Chris responded to our initial questions, as well as my second and third round of questions as she didn't quite make all the necessary changes in the protocol but rather inline only. Note I did make some changes yesterday in an attempt to be helpful and based on her responses to our questions inline. She has reviewed and is okay with the way I've revised things (Exp 001, Q5 and Q10a and removed Exp 002). Please let me know if you have any additional follow-up and I'll facilitate.
Best,
Preston

Response Submitted	Hampe, Christiane S.	8/18/2020 10:32 AM

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	Clarification by Pre-Reviewer Requested	Van Hooser, J.Preston	8/13/2020 4:35 PM

Hi Dr. Hampe,
I had a couple follow-up comments/questions based on your revision under the Reviewer Notes tab. Please review and edit/revise the pertinent sections of the protocol as necessary.
Best,
Preston

	Response Submitted	Hampe, Christiane S.	8/13/2020 1:36 PM

Obtained by Rise for Animals.
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Activity	Author	Activity Date
Clarification by Pre-Reviewer Requested	Van Hooser, J.Preston	8/13/2020 9:38 AM

Hi Dr. Hampe,
The veterinarian had some additional questions and I had a couple follow-up comments/questions as well under the Reviewer Notes tab. Please review and edit/revise the pertinent sections of the protocol as necessary. If you have any questions or need help navigating HoverBoard, please let me know.
Best,
Preston
jpvh@uw.edu
206-940-3176 (mobile)

Vet Consult Submitted	Stocking, Kim	8/12/2020 11:54 AM

Do you accept the submission? no

Vet Consult Sent	Stocking, Kim	8/12/2020 10:57 AM
Vet Consult Sent	Van Hooser, J.Preston	8/6/2020 7:02 PM
Private Comment Added	Van Hooser, J.Preston	8/6/2020 7:01 PM

Initial pre-review completed and group incorporated responses into revision. A few follow-up comments/questions but should be in good enough shape for vet consult.

Response Submitted	Hampe, Christiane S.	8/5/2020 12:55 PM

Thanks for the comments on our protocol.
We have responded to each of the comments.

Clarification by Pre-Reviewer Requested	Van Hooser, J.Preston	7/24/2020 6:52 PM

Hi Dr. Hampe,
The initial pre-review of your new protocol has been completed. There were several comments/questions in Reviewer Notes. Please review and edit/revise the pertinent sections of the protocol as necessary. Once I have your revision, I can forward it to the veterinarians for their required vet consult. If you have any questions or need help navigating HoverBoard, please let me know.
Best,
Preston
jpvh@uw.edu
206-940-3176 (mobile)

Tags Managed	Kunsman, Robyn	7/13/2020 8:29 AM
Tags Managed	Williams, Ashley E	7/10/2020 3:11 PM

PI completed AUMS

Coordinator Assigned	Van Hooser, J.Preston	7/10/2020 10:01 AM

Assigned to J.Preston Van Hooser

Coordinator Assigned	Jimenez, Selesteen	7/9/2020 1:08 PM

Assigned to OAW Blue Team

Tags Managed	Jimenez, Selesteen	7/9/2020 1:07 PM

Hold for PI aums

Submitted	Hampe, Christiane S.	7/9/2020 11:08 AM
Protocol Created	Hampe, Christiane S.	9/25/2019 2:59 PM

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Basic Information

1. *** Select research team:**
   Hampe
2. *** Title of protocol:**
   B lymphocyte tracking in mice.
3. *** Short title:**
   4189-04: B cell tracking
4. *** Summary of research:**
   Long-lasting drug-delivery remains a challenging aspect. We developed a cellular therapy, where autologous B lymphocytes are modified ex vivo to express the therapeutic protein, after which they are re-introduced in the patient. Because of the longevity of B lymphocytes, this treatment is expected to last several years and supply physiological amounts of the therapeutic protein throughout the life time of the cell.
   B lymphocytes are known to home to the bone marrow, but can also reside in other organs, namely the spleen, kidney, liver, the intestinal system and the CNS.
   Our goal is to establish the parameters involved in engraftment of transplanted B lymphocytes in the recipient mouse.
   Transplanted cells will be tracked using different methods. Engraftment and survival of B cells will be tracked in vivo by longitudinal detection of secreted alkaline phosphatase (SEAP) in the blood. Engrafted cells will be detected by GFP expression after euthanasia of the animals.
   The results of this research will be essential in the development of effective treatments for tissue-specific diseases that require long-time delivery of therapeutic proteins.
5. *** Principal investigator:**
   Christiane S. Hampe
6. *** What is the intention of the animal protocol?**
   Experimental Research

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Experimental Research Protocol Addition

1. * **Will the protocol include breeding?**
   ○ Yes ● **No**

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Protocol Team Members

1. **Identify each additional person involved in the design, conduct, or reporting of the research:**

Name	Role	Involved in Animal Handling	Authorized To Order Animals	E-mail	Phone
Christiane S. Hampe	Other	yes	yes	champe@uw.edu	+1 206 221-5275
Robert J Hunter	Research Scientist	yes	yes	bhunter@uw.edu	+1 206 685-3962
TRP/IVS Research Support	Research Scientist	yes	yes		

2. **If veterinary care will be provided by individuals outside of DCM or WaNPRC, provide the name, credentials and contact information below:**
N/A

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Funding Sources

1. Identify each organization supplying funding for the protocol:

	Funding Organization	eGC1 Number(s)
View	Other	N/A

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Scientific Aims

1. * Scientific aims of the research:

Determine engraftment sites of transplanted B cells in mice.

B lymphocytes are known to home to the bone marrow, but can also reside in other organs, namely the spleen, kidney, liver, the intestinal system and the CNS.

Our goal is to establish the parameters involved in engraftment of transplanted B lymphocytes in different tissues/organs. Expression of specific chemokine receptors aid in the homing of lymphocytes to different tissues/organs. We hypothesize that peripherally injected B lymphocytes cells carrying distinct chemokine receptors can be directed to target organs.

Tracking to transplanted B lymphocytes is essential to understand the fate of these cells and their ability to engraft permanently in target organs.

Transplanted cells will be tracked *in vivo* by presence of secreted alkaline phosphatase in blood and *in vitro* by detection of GFP-positive cells in different tissues after euthanasia.

The results of this research will be essential in the development of effective treatments for organ-specific diseases that require long-time delivery of therapeutic proteins.

2. * Using language understandable to non-scientists, describe the goals and significance of the protocol to humans, animals and science:

We developed a therapy, where the patient's own cells are modified to express the therapeutic, after which they are re-introduced in the patient. These cells survive for years in the body, so that this treatment is expected to last several years and supply the patient with the therapeutic for many years, rather than being dependent on lengthy and repeated treatments.
Our goal is to establish the conditions needed for survival of these cells in different organs/tissues. We propose that the injected cells can be guided to specific target organs, thus providing a viable option for treatment of organ-specific disorders. We are specifically interested in guiding the therapeutic cells to the brain in order to address brain disorders.
Tracking of these cells in the life animal is essential to understand the fate of these cells and their ability to survive permanently in the brain.
Transplanted cells can be tracked in vivo by the detection of a secreted biomarker and in vitro by the detection of labeled cells after euthanasia of the animal.
The results of this research will be essential in the development of effective treatments for organ-specific diseases that require long-time delivery of therapeutic proteins.

3. * Provide a statement to address the potential harm to the animals on this study (e.g., pain, distress, morbidity, mortality) relative to the benefits to be gained by performing the proposed work:

Animals are not expected to suffer harm in this study. However, the animals may experience some distress during blood sampling and/or injection.
The results coming out of the study will aid in the development of a novel therapeutic strategy to administer protein drug products for the long-term treatment of patients requiring life-long treatment such as in lysosomal storage diseases.

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Additional information regarding harm/benefit analyses may be found in an article by Graham and Prescott, 2015.

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Experiments

Note: If you will be administering cells, cell lines, sera or other biologicals to rodents, contact the Rodent Health Monitoring Program (RHMP, rhmp@uw.edu). Testing may be required prior to administration to rodents.

1. * Define the experiments to be used in this protocol:

Name	Species	USDA	Count	Count by Pain Category	Procedures	Husbandry Exception Types
001. B cell tracking	Mice	no	45	B: 0 C: 45 D: 0 E: 0	▪ Euthanasia: CO2 Followed by Secondary Methods (>10 days of age) (Standard) ▪ Substance Administration: Anesthesia, Isoflurane, Short Duration (<1 hour) (Standard) ▪ Substance Administration: Hampe: administration of B cells (Team)[Archived] ▪ Tissue/Blood Collection: Hampe: submandibular blood collection (Team) ▪ Tissue/Blood Collection: Blood Collection, Tail Prick (Standard)	Mice - No husbandry or enrichment exceptions.

2. Will any single animal undergo more than one survival surgery? (include any animal that underwent surgery prior to use on this protocol) ○ Yes ● **No**

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Procedure Personnel Assignment

1. * Select the team members who will be performing each procedure:

Procedure	Species	Is USDA Species	Team Members
Euthanasia: CO2 Followed by Secondary Methods (>10 days of age), ver. 2 (Standard)	Mice	no	TRP/IVS Research Support Christiane S. Hampe Robert J Hunter
Substance Administration: Anesthesia, Isoflurane, Short Duration (<1 hour), ver. 2 (Standard)	Mice	no	TRP/IVS Research Support Christiane S. Hampe Robert J Hunter
Substance Administration: Hampe: administration of B cells, ver. 1 (Team) [Archived]	Mice	no	TRP/IVS Research Support Christiane S. Hampe Robert J Hunter
Tissue/Blood Collection: Blood Collection, Tail Prick, ver. 2 (Standard)	Mice	no	TRP/IVS Research Support Christiane S. Hampe Robert J Hunter
Tissue/Blood Collection: Hampe: submandibular blood collection, ver. 1 (Team)	Mice	no	TRP/IVS Research Support Christiane S. Hampe Robert J Hunter

2. Team member training:

First Name Last Name Training

Christiane Hampe S.

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Rat Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	5/6/2014	

No experience data to display

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Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	7/10/2020	7/31/2023
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	3/8/2011	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	6/20/2017	6/20/2022
Annual DCM Facility Access Training (Rodent)	General	Online	Basic Course	Stage 1	3/1/2013	3/31/2014

Christiane Hampe S.

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Rat Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	5/6/2014	
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	7/10/2020	7/31/2023
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	3/8/2011	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	6/20/2017	6/20/2022
Annual DCM Facility Access Training (Rodent)	General	Online	Basic Course	Stage 1	3/1/2013	3/31/2014

No experience data to display

Robert J Hunter

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	12/12/2016	12/12/2021
Annual DCM Facility Access Training (Rodent)	General	Online	Basic Course	Stage 1	10/22/2020	10/31/2021
K-Wing Facility Orientation, ABSL-2 Users	Orientation	In Person	Basic Course	Stage 1	6/28/2013	

No experience data to display

Obtained by Rise for Animals.
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Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	12/20/2010	
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	9/6/2018	9/30/2021
Surgery Certification, Rodent - Waived	Surgery	Other	Basic Course	Stage 1	1/1/1950	
K-Wing Facility Orientation	Orientation	In Person	Basic Course	Stage 1	11/15/2006	
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	6/21/2013	
ARC Facility Orientation, Rodent Users	Orientation	In Person	Basic Course	Stage 1	8/29/2017	
T-Wing Facility Orientation	Orientation	In Person	Basic Course	Stage 1	11/13/2006	
Cervical Dislocation, Mouse Anesthetized	Procedure	In Person	Basic Course	Stage 1	12/20/2010	

TRP/IVS **Research Support**

No training data to display

No experience data to display

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Animal Details

1. *** How are animals acquired?**
   Purchased
2. **Describe the acquisition for:**
   a. **Not purchasing through DCM or WaNPRC:**
      N/A
3. **Identification of individual animals (other than cage cards):**
   a. **Method(s) (e.g., ear punch/tag, tattoo, tagging/banding, radio collar, etc.) (Note: If method is implantation (e.g. PIT tag), create or select an Implant procedure to describe the details. If method is surgical (e.g., satellite tag), create or select Survival Surgery procedure to describe the details):**
      Ear punch/tag.
   b. **Will external identification be replaced if it falls off/out? If yes, describe the plan for replacement:**
      Yes, ear punch/tag will be placed in the other ear.
   c. **Will external identification be removed as part of the protocol (e.g., radio collars on field animals)? If yes, describe the plan for removal:**
      No
4. **Identify strain/stock for rodents and genetically modified animals:**

	Species	Is USDA Species	Strain	Genetically Modified Strain	Phenotype Description
View	Mice	no	C57BL/6J, C57BL/6NTac, C57BL/6NCrl (C57BL/6)	no	None

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Animal Number Adjustments

"Animals Identified in Experiments" is the total number of animals per pain category listed in all experiments on this protocol. If more or fewer animals will be used on the protocol (see Help Text for examples), click Update to enter this new number in the corresponding "Adjusted Animal Count" column. **Only input numeric values in this field; 0 is acceptable.**
If no adjustment is required, the values in the "Animals Identified in Experiments" and "Adjusted Animal Count" columns must match. Click Update in each Pain Category row to input the matching value.
For questions about adjusting animal numbers, contact OAW.

1. * Click Update to adjust the number of animals to be used or produced for this protocol:

	Species	USDA Covered Species	Pain Category	Animals Identified in Experiments	Adjusted Animal Count
View	Mice	no	Pain Category B	0	0
View	Mice	no	Pain Category C	45	45
View	Mice	no	Pain Category D	0	0
View	Mice	no	Pain Category E	0	0

2. If you adjusted the number of animals for this protocol, explain why:

N/A

3. If you will be using animals to train personnel or to practice procedures included in this protocol, describe below:

N/A

4. Supporting documents:

Document Name	Date Modified
There are no items to display	

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Alternatives and Duplication Searches

Display Procedures that cause pain or distress: none

1. **Record all searches for any previous research that this protocol might duplicate:**

	Search Date	Searched Databases	Other
View	7/7/2020	PubMed/Medline	N/A

2. **Briefly describe the results of your searches and why you can or cannot incorporate the findings. Or, if a literature search was not performed, describe the methods used to determine that alternatives are not available or feasible:**

   No experiments using genetic engineering to guide B cells to specific tissues/organs have been found.

3. **Confirm that you have made every effort to ensure that this protocol is not unnecessary duplication of previous research:** ☑

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Housing and Use

Housing and use outside of the vivarium is not allowed without strong scientific justification.

1. Identify each location where animals will be housed:

	Facility	Species	Justification for Housing Outside Vivarium
View	ARCF ABSL1	Mice	N/A
View	ARCF ABSL1	Mice	N/A

2. Identify each location where animals will be used:

	Facility	Use	Species	Justification for Use Outside Vivarium
View	ARCF ABSL1	Administration of cells, anesthesia, blood draw, euthanasia.	Mice	N/A
View	ARCF ABSL1	Arrival prior to ABSL1.	Mice	N/A

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Disposition

1. **Disposition plans for the animals when this research is complete:**
   (check all that apply)
   Euthanasia
2. **If other, provide an animal disposition description:**
   N/A
3. **If protocol involves fixing tissues, list agents (e.g., paraformaldehyde, formalin):**
   10% formalin for histologic analysis.

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Refinement, Replacement and Reduction

1. Describe below how the three R's (refinement, replacement and reduction) have been employed on this project. Include alternatives that were considered for the procedures above that cause pain or distress:

* Refinement (use of methods to decrease animals' sensitivity to pain)

The animals will be anesthesized to reduce animal pain and/or distress during the study.

* Replacement (include in vitro tests, use of less sentient animals)

There are no non-animal alternatives available to investigate in vivo engraftment.

* Reduction (use of fewer animals to attain statistical significance)

Because of the nature of the planned study (pilot), we cannot carry out a statistical analysis to determine the fewest number of animals necessary.
We feel that 2 animals per timepoint is the minimum number of animals needed.

2. Describe the rationale for using animals and the appropriateness of the species proposed:

These studies examine the engraftment of B lymphocytes in recipient mice. Currently there is no animal-free model available to simulate mammalian immune reactions and cellular trafficking. Consequently, these studies can only be performed in vivo.

Mice are the appropriate species because they are the smallest mammalian species with available information regarding cellular trafficking and engraftment.

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Supporting Documents

1. **Attach supporting files:**

Document Name	Date Modified
There are no items to display	

Procedures Appendix:

Procedure Identification: Hampe: submandibular blood collection

1. *** Name of the procedure or surgery:**
   Hampe: submandibular blood collection
2. *** Select procedure type:**
   Tissue/Blood Collection
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

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Live Tissue/Blood Collection

1. *** Identify tissues to be collected:**
   Blood
2. **Describe timing and frequency of collection and amount to be collected:**

   Timing and frequency: baseline (prior to B cell injection), every 2 weeks until 12 weeks, or planned euthanasia, whichever occurs first.

   Maximum of 1% of body weight in a 2-week period.
3. **Select the anesthesia and analgesia procedures to be used:**

   Anesthesia, Isoflurane, Short Duration (<1 hour) Substance Administration 2 Standard
4. **If withholding anesthesia/analgesia when normally required, provide scientific justification:**
   N/A
5. **Describe any potential complications from collection:**
   N/A
6. *** Describe the collection procedure:**
   Submandibular blood sampling, using standard technique on awake or anesthetized mice.

Obtained by Rise for Animals.
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Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

Procedure Identification: Blood Collection, Tail Prick

1. *** Name of the procedure or surgery:**
   Blood Collection, Tail Prick
2. *** Select procedure type:**
   Tissue/Blood Collection
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

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Live Tissue/Blood Collection

1. *** Identify tissues to be collected:**
   Peripheral blood
2. **Describe timing and frequency of collection and amount to be collected:**
   See experiment for timing and frequency of collection. Total volume collected will not exceed 10 mL/kg within a 2 week period.
3. **Select the anesthesia and analgesia procedures to be used:**
   There are no items to display
4. **If withholding anesthesia/analgesia when normally required, provide scientific justification:**
   N/A
5. **Describe any potential complications from collection:**
   Scabbing of tail, vessel scarring over time
6. *** Describe the collection procedure:**
   The lateral tail vein is punctured with sterile needle or lancet for blood collection. Scab removal and/or gentle massage of the tail yields enough blood for subsequent sample collection. Additional vessel punctures may be needed.

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Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

HoverBoard

Procedure Identification: Anesthesia, Isoflurane, Short Duration (<1 hour)

1. *** Name of the procedure or surgery:**
   Anesthesia, Isoflurane, Short Duration (<1 hour)
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

   If yes,

   i. **Identify expected symptoms from administering this procedure:**
   N/A

   ii. **Identify criteria under which animals will be removed from research:**
   N/A

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Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Isoflurane	Standard	Inhalation	1-5%	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

The mouse is placed in an induction chamber and 1-5% isoflurane is administered until the mouse is recumbent. If more than momentary anesthesia is required, the mouse is removed from the chamber and positioned in a nose cone or intubated, with 1-5% isoflurane administered to maintain anesthesia. Adequate depth of anesthesia is monitored by respiratory rate, corneal reflex, and response to toe pinch. Heat support and eye lubrication will be provided.

3. Describe the intended effects of administering the substance(s):

General anesthesia

4. Describe any potential adverse reactions to administering the substance(s):

Respiratory depression, hypotension, cardiac arrhythmia

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

Isoflurane is administered using an anesthesia machine that has been adequately tested and certified.

Waste gas is scavenged using either an activated charcoal canister (e.g., F/Air), active scavenging system, or by conducting the work within a certified fume hood.

Isoflurane is an irritant and may cause reproductive problems in women. Refer to Occupational Health Recommendations.

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
There are no items to display	

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

View: Custom: Create Substance

1. *** Substance:**
   Isoflurane
2. **Route:**
   Inhalation

   If you indicated Other, specify the route:
   N/A
3. **Dose:**
   1-5%
4. **Frequency and duration of dosages:**
   Continuous for <1 hour (estimated)
5. **Volume (for rodents or intracranial injections):**
   N/A
6. **Concentration:**
   N/A
7. **Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:**
   Isoflurane is pharmaceutical grade.
8. **Complication remediation:**
   N/A
9. **Substance order for the procedure:**
   N/A

View: Custom SF: Procedure Identification

Procedure Identification: CO2 Followed by Secondary Methods (>10 days of age)

1. *** Name of the procedure or surgery:**
   CO2 Followed by Secondary Methods (>10 days of age)
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

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If yes,

i. **Identify expected symptoms from administering this procedure:**
N/A

ii. **Identify criteria under which animals will be removed from research:**
N/A

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Euthanasia

1. *** Method of euthanasia:**
   CO2 Overdose
2. **Describe procedure:**
   CO2 will be administered from a compressed commercial cylinder utilizing a flow meter to deliver 30-70% of the chamber volume/minute. Total gas exposure will be at least 5 minutes, with gas flow being maintained for at least 1 minute after apparent clinical death. A timer will be used to ensure adequate length of exposure.

   Secondary method of euthanasia will be one of the following: placed in a bag filled with CO2, decapitation, exsanguination, thoracotomy/tissue collection.
3. *** Will anesthesia be used?** Yes **No**
4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.
5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   Yes No

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

HoverBoard

Procedure Identification: Hampe: administration of B cells

1. *** Name of the procedure or surgery:**
   Hampe: administration of B cells
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

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Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	mouse B cells	Team	Intraperitoneal	107 cells	107 cells in 100µl-0.4ml	0.4 ml (maximum)	

2. * Describe step-by-step the procedure for administering the substance(s):

1. Restrain the animal and tilt so that the head is facing downward and its abdomen is exposed.
2. The site for an IP injection is the lower right quadrant of the abdominal cavity.
3. To prevent injection of intestine, only the tip of the needle should penetrate the abdominal wall.
4. Insert the sterile needle cranially into the abdominal cavity at a 30-45 degree angle caudal to the umbilicus and lateral to the midline. Inserting the needle on the mouse's right side avoids the cecum.
5. Aspirate prior to injecting material. If a brownish matter appears in the syringe, the needle is in the intestine or stomach. If a yellow fluid appears in the syringe, the needle is in bladder. In either case withdraw the needle and restart the procedure with a new needle. Animals will be euthanized after three unsuccessful IP injection attempts (i.e., penetration of intestine, stomach, or bladder).

Needle size – 25-27 g

3. Describe the intended effects of administering the substance(s):

Engraftment of B cells.

4. Describe any potential adverse reactions to administering the substance(s):

Accidental puncture of internal organs - animals will be monitored post-injection for hunched behavior, reduced activity, reluctant to move when touched. Animals demonstrating these signs will be euthanized.
We use C57Bl/6 donor mice and C57Bl/6 recipient mice and do not anticipate any adverse reaction to the administered cells.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA)**

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paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu**.**

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Procedure Documents

1. Supporting documents:

Document Name	Date Modified
There are no items to display	

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View: Custom: Create Substance

1. *** Substance:**
   mouse B cells
2. **Route:**
   Intraperitoneal

   If you indicated Other, specify the route:
3. **Dose:**
   107 cells
4. **Frequency and duration of dosages:**
   Once, 10 minutes
5. **Volume (for rodents or intracranial injections):**
   0.4 ml (maximum)
6. **Concentration:**
   107 cells in 100µl-0.4ml
7. **Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:**
   Agent is not available in pharmaceutical grade.
   Purification of mouse B cells, engineering and cell culturing are conducted under sterile conditions. B cell populations will be cryopreserved in cryostor 10 freezing medium at 1E8 cells /ml. B cell purity will be assessed by flow cytometry staining for CD3, CD19, CD20, CD56, and CD45 (pan).
8. **Complication remediation:**
   N/A
9. **Substance order for the procedure:**

Substances Appendix:

View: Custom SF: Substance Information

Substance Information: Isoflurane

1. *** Name:**
   Isoflurane
2. *** Substance types:** (select all that apply)
   Anesthetic
   Reproductive Hazard/Teratogen
3. *** Is this a hazardous agent:** **Yes** No

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NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

4. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom SF: Substance Information

Substance Information: mouse B cells

1. * Name:

mouse B cells

2. * Substance types: (select all that apply)

Cell, Cell Line, or Tissue - Other

3. * Is this a hazardous agent: Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

4. Supporting documents:

Document Name	Date Modified
There are no items to display	

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1. *** Select the funding organization:**
   Other

   If Other was selected in question 1, provide Funding Organization:
   Immusoft

2. *** All animal use projects must be reviewed for scientific merit prior to initiating animal use. Choose the required reviews for this project:**
   Has already been conducted and approved by a funding agency

3. **Provide name of the committee or the department reviewer** (Required if "Has been conducted by my department or school and has been found to be scientifically meritorious" was selected):

4. **eGC1 Number(s):**(assigned internally)
   N/A

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Experiments Appendix:

001. B cell tracking

1. *** Experiment name:**
   001. B cell tracking
2. *** Species:**
   Mice
3. **If other was selected, provide a species:**
4. **What is the scientific goal of this experiment:**
   We will test our hypothesis that B lymphocytes genetically engineered to express specific chemokine receptors are homing and engrafting to target tissues/organs.
5. *** Describe the animal experience in the experiment, from enrollment in the study to the final endpoint, including all procedures in chronological order and the minimum time between procedures. We encourage using bullet points, timeline, table, or a flow chart as appropriate:**

   1) Mice will be purchased from Jackson Laboratories and allowed to acclimate for 1 week at least.

   2) Some mice (donor) will be euthanized (see euthanasia procedure) and used for the preparation of B lymphocytes to be transplanted into recipient/experimental mice (see step 3).

   3) Experimental mice will be anesthesized (see anesthesia procedure), blood collected and B lymphocytes are injected intraperitoneal (see administration of B cells procedure).

   4) Every 2 weeks blood is collected from awake or anesthetized experimental mice (see tail prick or submandibular blood collection procedure). No more than 200µl of blood is collected at any time.

   5) Experimental mice are then euthanized at 1, 2, 4, 6, and 12 weeks (see euthanasia procedure) and the brain, lungs, liver, heart, and kidneys are harvested.

   Animal Sex:
   Female
   Male

   Animal Ages:
   8-10 weeks

   Animal Size:
   15-25 g

6. **Select experimental procedures:**

Name	Type	Version	Scope
CO2 Followed by Secondary Methods (>10 days of age)	Euthanasia	2	Standard

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Name	Type	Version	Scope
Anesthesia, Isoflurane, Short Duration (<1 hour)	Substance Administration	2	Standard
Hampe: administration of B cells	Substance Administration	1	Team
Blood Collection, Tail Prick	Tissue/Blood Collection	2	Standard
Hampe: submandibular blood collection	Tissue/Blood Collection	1	Team

7. **Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):**
Mice will be monitored 3 times a week for grooming, eating, defecation, locomotor activity. If animals show signs of distress or pain, such as hunched posture, scruffy fur, decreased activity, weight loss, dehydration, we will contact the veterinarians and consult with them. If the animal's distress or pain cannot be eased, or the animal lost more than 20% of its body weight, the animal will be euthanized.

8. **If there is expected mortality (spontaneous death) in this experiment:**

   a. Procedure/condition associated with mortality:
   N/A

   b. Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):
   N/A

   c. Explain why euthanasia is not possible or appropriate:
   N/A

9. **Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical signs to be monitored and criteria for euthanasia.**
No

10. *** Total number of animals used in this experiment:**(including all the animals to be produced)
45

    a. **Justify total number of animals used in this experiment:**

    Typical pharmacokinetic studies have a minimum of 2-3 animals per timepoint to account for some individual variation.

    Experimental Mice:

    We will use 5 time points (1, 2, 4, 6, and 12 weeks) for this longitudinal study, each with 2-3 animals per time point (possible variation between the animals can be adjusted for by measurements before and after). We are also requesting an inclusion of a repeat experiment in case our results are not conclusive. 5 time points x 3 animals x 2 = **30**

    Donor Mice (B cells):

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We will use one donor mouse for 2 recipient/experimental mice as a source of B cells to genetically engineer and inject IP based on our experience to carry out this longitudinal study. Total donor mice = **15**

11. Number of animals by pain and distress category:(include each animal only once in the highest pain category)

B: 0
C: 45
D: 0
E: 0

a. Justify the need for any animals in pain category E:

N/A

12. * Identify husbandry exceptions:

	Exception Type	Description and Justification
View	Mice - No husbandry or enrichment exceptions.	

13. Supporting documents:

Document Name	Date Modified
There are no items to display	

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1. * Exception type:

   Mice - No husbandry or enrichment exceptions.

2. Description and justification:

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1. * Identify the location where animals will be used:

ARCF ABSL1

a. For locations that are lab managed, provide justification for housing outside of the vivarium:

N/A

2. * What species will be housed in this location?

Common Name	Scientific Name
Mice	Mus

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Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

1. Campus:

Vivarium

2. Vivarium:

ARCF (Animal Research & Care Facility)

3. * BSL Level:

ARCF ABSL1

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

1. * Identify the location where animals will be used:

ARCF ABSL1

a. For locations that are lab managed, provide justification for housing outside of the vivarium:

N/A

2. * What species will be housed in this location?

Common Name	Scientific Name
Mice	Mus

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

View: UW IACUC Select Room Level

1. Campus:

Vivarium

2. Vivarium:

ARCF (Animal Research & Care Facility)

3. * BSL Level:

ARCF ABSL1

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

1. *** Identify the location where animals will be used:**

   ARCF ABSL1

   a. **For locations that are outside of the vivarium, provide justification for the use of this space:**

      N/A

2. *** What species will be used in this location?**

Common Name	Scientific Name
Mice	Mus

3. **Describe how this location will be used:**

   Administration of cells, anesthesia, blood draw, euthanasia.

4. *** If animals are left unattended in this location, provide an explanation and include maximum duration:**

   N/A

5. **Describe how animals will be transported to and from this location, including container and route. (Note: use of private vehicles requires IACUC approval):**

   N/A

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1. Campus:

Vivarium

2. Vivarium:

ARCF (Animal Research & Care Facility)

3. * BSL Level:

ARCF ABSL1

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1. *** Identify the location where animals will be used:**

   ARCF ABSL1

   a. **For locations that are outside of the vivarium, provide justification for the use of this space:**

      N/A

2. *** What species will be used in this location?**

Common Name	Scientific Name
Mice	Mus

3. **Describe how this location will be used:**

   Arrival prior to ABSL1.

4. *** If animals are left unattended in this location, provide an explanation and include maximum duration:**

   Animals will not be left unattended.

5. **Describe how animals will be transported to and from this location, including container and route. (Note: use of private vehicles requires IACUC approval):**

   N/A

Obtained by Rise for Animals.
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View: UW IACUC Select Room Level

1. Campus:

Vivarium

2. Vivarium:

ARCF (Animal Research & Care Facility)

3. * BSL Level:

ARCF ABSL1

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

From: "Molly K. Lucas" <mklucas@uw.edu>
To: "Nicholas L. Reyes" <nlreyes@uw.edu>
Sent: 8/19/2020 12:21:15 PM
Subject: another Zee Liu protocol Q

Hi Nick,

It turns out I saw another thing that was missing - both survival surgeries were lacking detail re: closures.

In response to my comment asking for more detail (and giving our recommendation of synthetic non-abs for non-buried sutures and synthetic absorbable for buried), he added this to the implant surgery: "Finally, the submandibular incision will be closed with synthetic non-absorbable for non-buried skin sutures."

And this to the tongue reduction surgery (which will have a skin incision overlying the masseter muscle in addition to the tongue): "Finally, the incision will be closed with synthetic non-absorbable for non-buried skin sutures without layers." for the skin, and this for the tongue, " The lesion will be closed with absorbable sutures in 2-3 layers (See Fig. 3: Coblation)."

I'm thinking there will need to be at least more internal layer for the submandibular (muscle and/or SC in addition to skin) - what do you think? And what do you think about the tongue plan?

I think if we suggest a plan (with specific language), he will probably accept it... at least that's how it's been working for my other suggestions. I've been doing more direct editing than I normally do. These surgeries are just a little more difficult for me to envision compared to a laparatomy or something.

Please let me know what you think, e.g., good language to include for the closures (I usually expect it to say how many layers and general type of suture, e.g., "synthetic non-absorbable," but does not need to list suture size).

Thanks,
Molly

Link to protocol: https://hoverboard.washington.edu/Hoverboard/sd/Rooms/DisplayPages/LayoutInitial?Container=com.webridge.entity.Entity[OID[BBD9E52211666B4AB9BA57EE24AED8CC]]

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Wednesday, August 19, 2020 12:21 PM
To: Nicholas L. Reyes
Subject: another Zee Liu protocol Q

Hi Nick,

It turns out I saw another thing that was missing - both survival surgeries were lacking detail re: closures.

In response to my comment asking for more detail (and giving our recommendation of synthetic non-abs for non-buried sutures and synthetic absorbable for buried), he added this to the implant surgery: "Finally, the submandibular incision will be closed with synthetic non-absorbable for non-buried skin sutures."

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Please let me know what you think, e.g., good language to include for the closures (I usually expect it to say how many layers and general type of suture, e.g., "synthetic non-absorbable," but does not need to list suture size).

Thanks,
Molly

Link to protocol: https://hoverboard.washington.edu/Hoverboard/sd/Rooms/DisplayPages/LayoutInitial?Container=com.webridge.entity.Entity[OID[BBD9E52211666B4AB9BA57EE24AED8CC]]

Obtained by Rise for Animals.
Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021

From: Molly K. Lucas <mklucas@uw.edu>
Sent: Friday, July 31, 2020 2:49 PM
To: Michelle Brot
Subject: Automatic reply: Zi-Liu protocol

Hello,

I will be out of the office 7/30 and 7/31/20, without email access. I will respond as soon as possible when I return.

Molly

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Uploaded to Animal Research Laboratory Overview (ARLO) on 05/13/2021